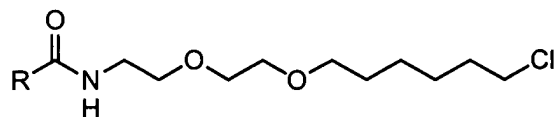


**In the Claims**

Please amend the claims as follows:

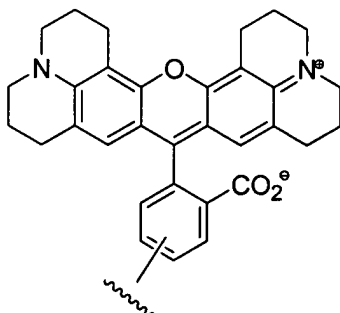
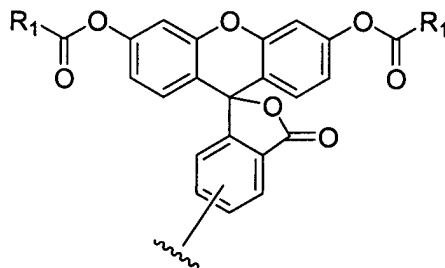
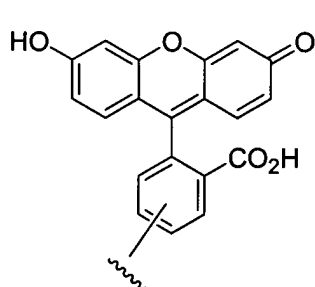
1. (Currently Amended) A compound of formula (I): R-linker-A-X, wherein R is one or more functional groups, ~~wherein the linker is a multiatom straight or branched chain including C, N, S, or O~~ wherein the linker is a divalent branched or unbranched carbon chain comprising from about 2 to about 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A is (CH<sub>2</sub>)<sub>n</sub> and n = 4-10, wherein A-X is a substrate for a dehalogenase, and wherein X is a halogen.
2. (Original) The compound of claim 1 which is a substrate for a *Rhodococcus* dehalogenase.
3. (Original) The compound of claim 1 wherein X is Cl or Br.
4. (Canceled)
5. (Original) The compound of claim 1 wherein the linker comprises (CH<sub>2</sub>CH<sub>2</sub>O)<sub>y</sub> and y = 2-8.
6. (Original) The compound of claim 1 wherein the linker separates R and A by at least 12 atoms.
7. (Original) The compound of claim 1 wherein the linker comprises 3 to 30 atoms.
8. (Original) The compound of claim 1 wherein the linker has 11 to 30 atoms.

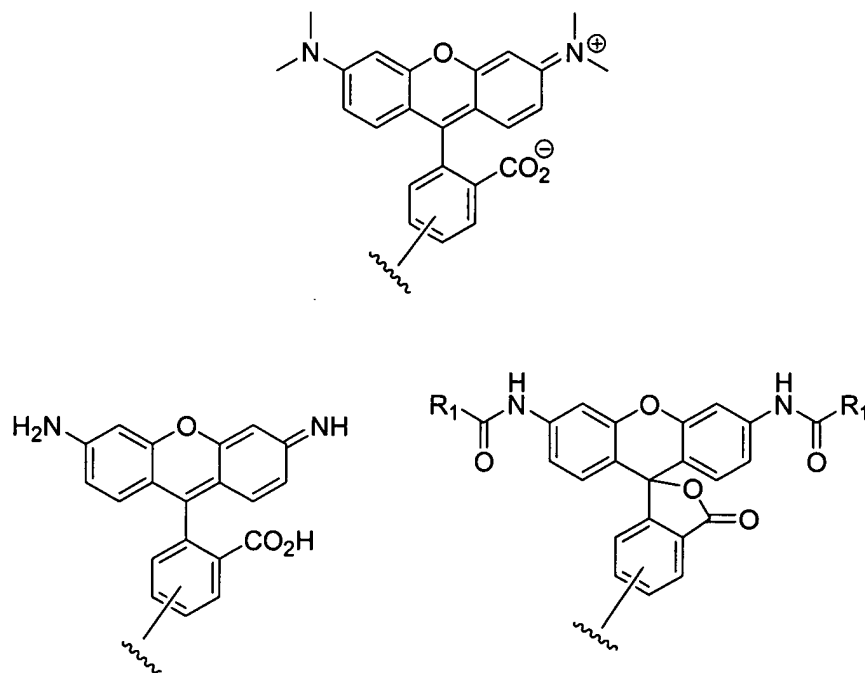
9. (Original) The compound of claim 1 which is



10. (Original) The compound of claim 1 wherein at least one functional group is an amino acid, protein, glycoprotein, nucleic acid molecule, drug, lipid, biotin, or solid support.
11. (Original) The compound of claim 1 wherein at least one functional group is an optically detectable molecule.
12. (Withdrawn) The compound of claim 11 wherein at least one functional group is a fluorophore.

13. (Withdrawn) The compound of claim 1 wherein R is one of





and wherein  $\text{R}_1$  is  $\text{C}_1\text{-C}_8$ .

14. (Withdrawn) The compound of claim 1 which comprises two functional groups.
15. (Original) The compound of claim 1 wherein at least one functional group binds  $\text{Ca}^{2+}$ , binds  $\text{K}^+$ , binds  $\text{Na}^+$ , is pH sensitive, is a radionuclide, is electron opaque, is a chromophore, is a MRI contrast agent, fluoresces in the presence of NO or is sensitive to a reactive oxygen.
16. (Withdrawn) A mutant dehalogenase comprising at least one amino acid substitution relative to a corresponding wild-type dehalogenase, wherein the mutant dehalogenase forms a bond with a dehalogenase substrate which comprises one or more functional groups, which bond is more stable than the bond formed between the corresponding wild-type dehalogenase and the substrate, wherein the at least one amino acid substitution in the mutant dehalogenase is a substitution at an amino acid residue in the corresponding wild-type dehalogenase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type dehalogenase and the substrate or at an amino acid residue in the corresponding wild-type dehalogenase that forms an ester

intermediate with the substrate, wherein the substituted amino acid at a residue associated with activating a water molecule is not glutamine or asparagine.

17. (Withdrawn) The mutant dehalogenase of claim 16 wherein the substitution is at a residue in the wild-type dehalogenase that activates the water molecule.
18. (Withdrawn) The mutant dehalogenase of claim 17 wherein the residue in the wild-type dehalogenase that activates the water molecule is histidine.
19. (Withdrawn) The mutant dehalogenase of claim 16 wherein the substitution is at a residue in the wild-type dehalogenase which forms an ester intermediate with the substrate.
20. (Withdrawn) The mutant dehalogenase of claim 19 wherein the residue in the wild-type dehalogenase which forms the ester intermediate is aspartate.
21. (Withdrawn) The mutant dehalogenase of claim 16 wherein the at least one substitution is at a position corresponding to amino acid residue 272 of a *Rhodococcus rhodochrous* dehalogenase.
22. (Withdrawn) The mutant dehalogenase of claim 21 wherein the substituted amino acid at the position corresponding to amino acid residue 272 is phenylalanine, glycine or alanine.
23. (Withdrawn) The mutant dehalogenase of claim 16 wherein the at least one substitution is at a position corresponding to amino acid residue 106 of a *Rhodococcus rhodochrous* dehalogenase.
24. (Withdrawn) The mutant dehalogenase of claim 23 wherein the substituted amino acid at the position corresponding to amino acid residue 106 is cysteine or glutamate.

25. (Withdrawn) The mutant dehalogenase of claim 16 further comprising a protein of interest, thereby yielding a fusion protein.
26. (Withdrawn) The mutant dehalogenase of claim 25 wherein the protein of interest is a selectable marker protein, membrane protein, cytosolic protein, nuclear protein, structural protein, an enzyme, an enzyme substrate, a receptor protein, a transporter protein, a transcription factor, a channel protein, a phospho-protein, a kinase, a signaling protein, a metabolic protein, a mitochondrial protein, a receptor associated protein, a nucleic acid binding protein, an extracellular matrix protein, a secreted protein, a receptor ligand, a serum protein, an immunogenic protein, a fluorescent protein, or a protein with reactive cysteines.
27. (Withdrawn) The mutant dehalogenase of claim 16 which has at least 85% amino acid sequence identity to the corresponding wild-type dehalogenase.
- 28-34. (Canceled)
35. (Currently Amended) A method to detect or determine the presence or amount of a mutant hydrolase, comprising:
- a) contacting a mutant hydrolase with a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the mutant hydrolase is a mutant dehalogenase and wherein

the substrate is a compound of formula (I): linker R-linker-A-X, wherein the linker is a divalent branched or unbranched carbon chain comprising from about 2 to about 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is (CH<sub>2</sub>)<sub>n</sub> and n = 4-10, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker; and

- b) detecting or determining the presence or amount of the functional group, thereby detecting or determining the presence or amount of the mutant hydrolase.
36. (Withdrawn) The method of claim 35 wherein the substitution is at a residue in the wild-type hydrolase that activates the water molecule.
37. (Withdrawn) The method of claim 36 wherein the residue in the wild-type hydrolase that activates the water molecule is histidine.
38. (Withdrawn) The method of claim 35 wherein the substitution is at a residue in the wild-type hydrolase that forms an ester intermediate with the substrate.
39. (Withdrawn) The method of claim 38 wherein the residue in the wild-type hydrolase that forms an ester intermediate with the substrate is aspartate.
40. (Currently Amended) A method to isolate a molecule, cell or subcellular organelle of interest in a sample, comprising:
- a) contacting a sample with a fusion protein comprising a mutant hydrolase and a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant

hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the fusion protein comprises a protein which binds a molecule, cell or subcellular organelle of interest, wherein the mutant hydrolase is a mutant dehalogenase and wherein the substrate is a compound of formula (I): R-linker-A-X, wherein the linker is a divalent branched or unbranched carbon chain comprising from about 2 to about 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is (CH<sub>2</sub>)<sub>n</sub> and n = 4-10, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker; and  
b) isolating the molecule, cell or subcellular organelle of interest.

41. (Withdrawn) The method of claim 40 wherein the substitution is at a residue in the wild-type hydrolase that activates the water molecule.
42. (Withdrawn) The method of claim 41 wherein the residue in the wild-type hydrolase that activates the water molecule is histidine.
43. (Withdrawn) The method of claim 40 wherein the substitution is at a residue in the wild-type hydrolase that forms an ester intermediate with the substrate.
44. (Withdrawn) The method of claim 43 wherein the residue in the wild-type hydrolase that forms an ester intermediate with the substrate is aspartate.

45. (Withdrawn) The method of claim 40 wherein at least one functional group is a solid support or a molecule which binds to a solid support.
46. (Withdrawn) The method of claim 40 wherein the molecule of interest is a protein.
47. (Currently Amended) A method to label a cell, comprising:
- a) contacting a cell comprising a mutant hydrolase with a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves a bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the mutant hydrolase is a mutant dehalogenase and wherein the substrate is a compound of formula (I): R-linker-A-X, wherein the linker is a divalent branched or unbranched carbon chain comprising from about 2 to about 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is (CH<sub>2</sub>)<sub>n</sub> and n = 4-10, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker; and
  - b) detecting or determining the presence or amount of the functional group.

48. (Withdrawn) The method of claim 47 wherein the substitution is at a residue in the wild-type hydrolase that activates the water molecule.
49. (Withdrawn) The method of claim 48 wherein the residue in the wild-type hydrolase that activates the water molecule is histidine.
50. (Withdrawn) The method of claim 47 wherein the substitution is at a residue in the wild-type hydrolase that forms an ester intermediate with the substrate.
51. (Withdrawn) The method of claim 50 wherein the residue in the wild-type hydrolase that forms an ester intermediate with the substrate is aspartate.
52. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein the wild-type hydrolase is a dehalogenase.
- 53-54. (Canceled)
55. (Currently Amended) The method of claim ~~53~~ 52 wherein the linker comprises  $(\text{CH}_2\text{CH}_2)_y$  and  $y = 2-8$ .
56. (Currently Amended) The method of claim ~~53~~ 52 wherein the linker separates R and A by at least 12 atoms.
57. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein the wild-type hydrolase is a serine beta-lactamase.
58. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein the mutant hydrolase is present in a cell or on the surface of a cell.

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59. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein at least one functional group is an amino acid, protein, glycoprotein, nucleic acid molecule, drug, lipid, biotin, or solid support.
60. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein at least one functional group is an optically detectable molecule.
61. (Withdrawn) The method of claim 60 wherein at least one functional group is a fluorophore.
62. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein the substrate comprises two functional groups.
63. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein at least one functional group binds  $\text{Ca}^{2+}$ , binds  $\text{K}^{+}$ , binds  $\text{Na}^{+}$ , is pH sensitive, is electron opaque, is a chromophore, is a MRI contrast agent, is a radionuclide, fluoresces in the presence of NO or is sensitive to a reactive oxygen.
64. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein the presence of at least one functional group in a cell is correlated to the subcellular location of the mutant hydrolase.
65. (Withdrawn) The method of any one of claims 35, 40 or 47 wherein the mutant hydrolase further comprises a protein of interest, thereby yielding a fusion protein.
66. (Withdrawn) The method of claim 65 wherein the protein of interest is a selectable marker protein, membrane protein, cytosolic protein, nuclear protein, structural protein, an enzyme, an enzyme substrate, a receptor protein, a transporter protein, a transcription factor, a channel protein, a phospho-protein, a kinase, a signaling protein, a metabolic protein, a mitochondrial protein, a receptor associated protein, a nucleic acid binding

protein, an extracellular matrix protein, a secreted protein, a receptor ligand, a serum protein, an immunogenic protein, a fluorescent protein, or a protein with reactive cysteine.

67. (Withdrawn) The method of claim 47 wherein the mutant hydrolase further comprises a selectable marker protein.
68. (Withdrawn) The method of claim 67 wherein at least one functional group in the substrate is a fluorophore.
69. (Withdrawn) The method of claim 68 wherein the mutant hydrolase forms an ester bond with the substrate.
70. (Withdrawn) The method of claim 68 wherein the mutant hydrolase forms a thioester bond with the substrate.
71. (Withdrawn) The method of claim 47 further comprising contacting the cell with a fixative prior to or after contacting the cell with the substrate.
72. (Withdrawn) The method of claim 47 further comprising contacting the cell with a fixative concurrently with contacting the cell with the substrate.
73. (Withdrawn) The method of claim 71 or 72 wherein the cell is fixed with methanol, acetone and/or paraformaldehyde.
74. (Withdrawn) The method of claim 67 further comprising contacting the cell with a fixative prior to or after contacting the cell with the substrate.
75. (Withdrawn) The method of claim 67 further comprising contacting the cell with a fixative concurrently contacting the cell with the substrate.

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76. (Withdrawn) The method of claim 74 or 75 wherein the cell is fixed with methanol, acetone and/or paraformaldehyde.
77. (Withdrawn) The method of claim 52 wherein the mutant dehalogenase is encoded by a nucleic acid sequence which is optimized for expression in a selected host cell.
78. (Previously Presented) The compound of claim 1 having formula II, formula III, formula IV, formula V, formula VI, formula VII, formula VIII, formula IX, formula X, formula XI, formula XII, formula XIII, formula XIV, formula XV, formula XVI, formula XVII, formula XVIII, formula XIX, formula XX, formula XXI, formula XXII, formula XXIII, formula XXIV, formula XXV, formula XXVI, formula XXVII, or formula XXVIII
- 79-86. (Canceled)
87. (Withdrawn) An isolated cell comprising a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a selectable marker protein and a protein which is capable of irreversibly or stably binding a substrate or a portion thereof which includes a functional group.
88. (Withdrawn) The cell of claim 87 wherein the functional group is a fluorophore.
89. (Withdrawn) The isolated cell of claim 87 wherein the protein which is capable of stably binding a substrate which includes a functional group is a mutant hydrolase comprising at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the mutant hydrolase forms a bond with a hydrolase substrate which comprises a fluorophore, which bond is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond

formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate.

90. (Withdrawn) The isolated cell of claim 87 wherein the protein irreversibly binds at least a portion of the substrate which includes the functional group.
91. (Withdrawn) A method to label a cell, comprising:  
contacting cells comprising a fusion protein comprising a selectable marker protein and a second protein which is capable of irreversibly or stably binding a substrate or a portion thereof which includes a functional group, with the substrate.
92. (Withdrawn) The method of claim 91 wherein cells which express the selectable marker protein are selected prior to contacting the cells with the substrate.
93. (Withdrawn) The method of claim 91 wherein cells which express the selectable marker protein are selected after contacting the cells with the substrate.
94. (Withdrawn) The method of claim 91 wherein the functional group is a fluorophore.
95. (Withdrawn) The method of claim 91 further comprising contacting the cells with a fixative prior to or after contacting the cells with the substrate.
96. (Withdrawn) The method of claim 91 further comprising contacting the cells with a fixative concurrently with contacting the cells with the substrate.
97. (Withdrawn) A mutant hydrolase comprising at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the mutant hydrolase forms a bond with a hydrolase substrate which comprises one or more functional groups, which bond is more stable than the bond formed between the corresponding wild-type hydrolase and the

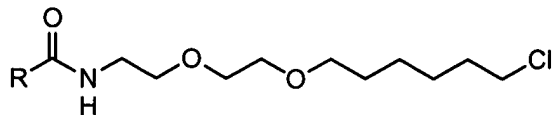
substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the substituted amino acid at a residue associated with activating a water molecule is not glutamine or asparagine.

98. (Withdrawn) The mutant hydrolase of claim 97 wherein the substitution is at a residue in the wild-type hydrolase that activates the water molecule.
99. (Withdrawn) The mutant hydrolase of claim 98 wherein the residue in the wild-type hydrolase that activates the water molecule is histidine.
100. (Withdrawn) The mutant hydrolase of claim 97 wherein the substitution is at a residue in the wild-type hydrolase which forms an ester intermediate with the substrate.
101. (Withdrawn) The mutant hydrolase of claim 100 wherein the residue in the wild-type hydrolase which forms the ester intermediate is aspartate.
102. (Withdrawn) The mutant hydrolase of claim 97 wherein the at least one substitution is at a position corresponding to amino acid residue 272 of a *Rhodococcus rhodochrous* dehalogenase.
103. (Withdrawn) The mutant hydrolase of claim 102 wherein the substituted amino acid at the position corresponding to amino acid residue 272 is phenylalanine or glycine.
104. (Withdrawn) The mutant hydrolase of claim 97 wherein the at least one substitution is at a position corresponding to amino acid residue 106 of a *Rhodococcus rhodochrous* dehalogenase.

105. (Withdrawn) The mutant hydrolase of claim 104 wherein the substituted amino acid at the position corresponding to amino acid residue 106 is cysteine or glutamate.
106. (Withdrawn) The mutant hydrolase of claim 97 wherein the substituted amino acid at a residue associated with activating a water molecule is not methionine, aspartate, or alanine.
107. (Currently Amended) A method for preparing a compound of formula R-Linker-A-X comprising coupling a compound of formula R-Y with a compound of formula Z-Linker-A-X, wherein Y and Z are groups that can react to link R- to -Linker-A-X, wherein the linker is a divalent branched or unbranched carbon chain comprising from about 2 to about 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is (CH<sub>2</sub>)<sub>n</sub> and n = 4-10, wherein X is a halogen, and wherein R is a biotin functional group that is capable of being coupled through its carboxy terminus to the linker.
108. (Original) The method of claim 107 wherein R-Y is an activated ester of a compound of formula R and wherein Z is an amine suitable to react with the activated ester to form an amide bond.
109. (Original) A method for preparing a compound of formula R-Linker-A-X wherein the Linker comprises an amide bond comprising coupling a corresponding activated ester with a corresponding amine to provide the compound of formula R-Linker-A-X.
110. (New) The compound of claim 1 wherein R is a biotin functional group coupled through its carboxy terminus to the linker.

111. (New) The compound of claim 110 which is a substrate for a *Rhodococcus* dehalogenase.
112. (New) The compound of claim 110 wherein X is Cl or Br.
113. (New) The compound of claim 110 wherein the linker comprises 3 to 30 atoms.
114. (New) The compound of claim 110 wherein the linker has 11 to 30 atoms.
115. (New) The compound of claim 110 which is N-{2-[2-(6-Chlorohexyloxy)-ethoxy]-ethyl}-biotin-amide.
116. (New) The compound of claim 110 wherein R is separated from A-X by up to 100 angstroms.
117. (New) The compound of claim 110 wherein R is separated from A-X by up to 500 angstroms.
118. (New) The compound of claim 110 wherein the chain comprises (CH<sub>2</sub>CH<sub>2</sub>O)<sub>y</sub> and y = 2-8.

119. (New) A compound prepared by the method of claim 107 wherein the compound is



120. (New) A compound of formula (I): R-linker-A-X, wherein R is one or more functional groups, wherein the linker is a divalent branched or unbranched carbon chain comprising from about 2 to about 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is

optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A is  $(CH_2)_n$  and  $n = 2-10$ , wherein A-X is a substrate for a dehalogenase, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker.